MycoKeys 87: 77–98 (2022)
doi: 10.3897/mycokeys.87.79433
https://mycokeys.pensoft.net

RESEARCH ARTICLE

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MycoKeys
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Pleocatenata chiangraiensis gen. et. sp. nov. (Pleosporales, Dothideomycetes) from medicinal plants in northern Thailand

Ya-Ru Sun[1,2,3], Ning-Guo Liu[2,5], Kevin D. Hyde[2,3,4], Ruvishika S. Jayawardena[2,3], Yong Wang[1]

1 *Department of Plant Pathology, College of Agriculture, Guizhou University, Guiyang 550025, China* **2** *Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand* **3** *School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand* **4** *Innovative Institute of Plant Health, Zhongkai University of Agriculture and Engineering, Haizhu District, Guangzhou 510000, China* **5** *School of Life Science and Technology, Center for Informational Biology, University of Electronic Science and Technology of China, Chengdu 611731, China*

Corresponding author: Yong Wang (yongwangbis@aliyun.com)

Academic editor: Nalin Wijayawardene | Received 16 December 2021 | Accepted 19 January 2022 | Published 11 February 2022

Citation: Sun Y-R, Liu N-G, Hyde KD, Jayawardena RS, Wang Y (2022) *Pleocatenata chiangraiensis* gen. et. sp. nov. (Pleosporales, Dothideomycetes) from medicinal plants in northern Thailand. MycoKeys 87: 77–98. https://doi.org/10.3897/mycokeys.87.79433

Abstract

Pleocatenata, a new genus, is introduced with its type species, *Pleocatenata chiangraiensis*, which was isolated from withered twigs of two medicinal plants, *Clerodendrum quadriloculare* (Blanco) Merr (Verbenaceae) and *Tarenna stellulata* (Hook.f.) Ridl (Rubiaceae) in northern Thailand. The genus is characterized by mononematous, septate, brown or dark brown conidiophores, monotretic conidiogenous cells and catenate, obclavate, olivaceous to blackish brown conidia. Phylogenetic analysis of combined LSU, SSU, *tef1-α*, *rpb2* and ITS sequence data showed *Pleocatenata* forms a distinct phylogenetic lineage in Pleosporales, Dothideomycetes. Therefore, we treat *Pleocatenata* as Pleosporales genera *incertae sedis* based on morphology and phylogenetic analyses. Descriptions and illustrations of the new taxa are provided, and it is compared with morphologically similar genera.

Keywords

Genera *incertae sedis*, hyphomycetes, multi-gene phylogeny, taxonomy

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Introduction

Medicinal plants are a rich source of natural products with biological and chemical properties. They are used in health care or treatment of human ailments and have been used since prehistoric times worldwide (Rasool-Hassan 2012). Many fungi have been found on medicinal plants and are members of Dothideomycetes and Sordariomycetes (Bhagat et al. 2012; Long et al. 2019; Ma et al. 2019; Hyde et al. 2020; Tennakoon et al. 2021). They form important associations with medicinal plants and as pathogens or saprobes (Long et al. 2019; Tennakoon et al. 2021), sources of medicines (Strobel et al. 1993; Huang et al. 2008; Hyde et al. 2019), involved in nutrient recycling (Bonnardeaux et al. 2007) and some are used in biological control (Hyde et al. 2019).

Pleosporales is the largest order in Dothideomycetes, which accounts for about a quarter of the class (Zhang et al. 2012; Hyde et al. 2013; Hongsanan et al. 2020a). They have a worldwide distribution with diverse lifestyles, including saprobes, pathogens of plants and humans, endophytes, epiphytes and hyperparasites (Ramesh 2003; Kirk et al. 2008; Zhang et al. 2012; Hyde et al. 2013; Sun et al. 2019; Ferdinandez et al. 2021). Many species in *Alternaria* Nees, *Curvularia* Boedijn and *Corynespora* Güssow, can invade medicinal plants and cause leaf spots and other diseases, as economically important plant pathogens (Mathiyazhagan et al. 2004; Abtahi and Nourani 2017; Zhang et al. 2020), and some also pose a threat to human health (Hyde et al. 2018; Iturrieta-González et al. 2020). Endophytes in Pleosporales also show important biocontrol value (Su et al. 2014; De Silva et al. 2019; Hyde et al. 2019), for example, an extract from *Cochliobolus spicifer* R.R. Nelson has mosquito-larvicidal activity (Abutaha et al. 2015).

The sexual morph of Pleosporales is characterized by uniloculate ascomata typically with papillae, ostioles and pseudoparaphyses, generally fissitunicate asci bearing mostly septate ascospores of different colours and shapes (Ramesh 2003; Kirk et al. 2008; Zhang et al. 2012; Hyde et al. 2013). Coelomycetes and hyphomycetes are the asexual morphs of pleosporalean taxa (Zhang et al. 2012; Hongsanan et al. 2020a). Recent comprehensive studies on Dothideomycetes treated 91 families in Pleosporales (Hongsanan et al. 2020a). More than 40 genera are recognized as genera *incertae sedis* in Pleosporales (Hongsanan et al. 2020a; Wijayawardene et al. 2020, 2021). This uncertainty in genetic placement occurs for the following reasons: 1) some genera lack sufficient collections even though molecular data is available, they are not included in any families in phylogenetic analyses, eg. *Aegeanispora* E.B.G. Jones & Abdel-Wahab, *Antealophiotrema* A. Hashim. & Kaz. Tanaka and *Perthomyces* Crous (Li et al. 2016; Abdel-Wahab et al. 2017; Crous et al. 2017); 2) due to the diverse morphology of hyphomycetous asexual morphs, it is difficult to determine their familial placement without the sexual morph and molecular data. Examples are *Briansuttonia* R.F. Castañeda, Minter & Saikawa, *Cheiromoniliophora* Tzean & J.L. Chen, *Dangeardiella* Sacc. & P. Syd and *Pleosphaerellula* Naumov & Czerepan (Obrist 1959; Tóth 1975; Tzean and Chen 1990; Castañeda-Ruiz et al. 2004).

During the examination of collections from medicinal plants in northern Thailand (Sun et al. 2021), two isolates representing a new species were obtained from

Clerodendrum quadriloculare and *Tarenna stellulata.* Morphology and phylogenetic analyses confirmed that it was distinct in Pleosporales, but its familial placement was uncertain. Thus, we introduced a new genus, *Pleocatenata* (Pleosporales, genera *incertae sedis*) to accommodate the new species, *P. chiangraiensis*.

Materials and methods

Collection, examination and isolation

The isolates used in this study were collected from decaying twigs of *Clerodendrum quadriloculare* and *Tarenna stellulata* from Mae Fah Luang University, Chiang Rai, Thailand during June to July 2020 in terrestrial habitat. The samples were packaged in envelopes and returned to the laboratory as described in Senanayake et al. (2020). The fruiting bodies on natural substrates were observed and photographed using a stereo-microscope (SteREO Discovery, V12, Carl Zeiss Microscopy GmBH, Germany). Morphological characters were observed using a Nikon ECLIPSE Ni compound microscope (Nikon, Japan) and photographed with a Nikon DS-Ri2 digital camera (Nikon, Japan). The Adobe Photoshop CS6 Extended v. 13.0 software was used to make photo-plates. Measurements were done with the Tarosoft (R) Image Frame Work software.

Single spore isolations were used to obtain pure cultures following the methods described by Senanayake et al. (2020). Germinated conidia were transferred to new potato dextrose agar (PDA) plates and incubated at 26 °C for four weeks. The pure cultures obtained were deposited in Mae Fah Luang University Culture Collection (MFLUCC), Chiang Rai, Thailand. Herbaria materials were deposited in the herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand. Facesoffungi (FoF) and Index Fungorum numbers were acquired as described in Jayasiri et al. (2015) and Index Fungorum (2022).

DNA extraction, PCR amplification and sequencing

Fresh fungal mycelia grown on PDA medium for 4 weeks at 26 °C were scraped with a sterile scalpel. Genomic DNA was extracted from scraped mycelia using the BIOMIGA Fungus Genomic DNA Extraction Kit (GD2416, BIOMIGA, San Diego, California, USA) following the manufacture's protocol. Five genes were selected in this study: the 28S subunit rDNA (LSU), the 18S subunit rDNA (SSU), the internal transcribed spacers (ITS), the translation elongation factor 1 (*tef1-α*), and the RNA polymerase II subunit 2 (*rpb2*). Polymerase chain reaction (PCR) was carried out in 20 µL reaction volume which contained 10 µL 2 × PCR Master Mix, 7 µL ddH_2O, 1 µL of each primer, and 1 µL template DNA. The PCR thermal cycle program and primers are given (Table 1). Purification and sequencing of PCR products were carried out at SinoGenoMax (Beijing) Co., China.

Table 1. Primers and PCR procedures used in this study.

Locus	Primers		PCR procedures	References
	Name	Sequence (5'–3')		
Large subunit (LSU)	LR0R	ACCCGCTGAACTTAAGC	94 °C 3 min; 35 cycles of 94 °C 30 s, 52 °C 30 s, 72 °C 1 min; 72 °C 8 min; 4 °C on hold	Vilgalys and Hester (1990), Rehner and Samuels (1994)
	LR5	TCCTGAGGGAAACTTCG		
Small subunit (SSU)	NS1	GTAGTCATATGCTTGTCTC		White et al. (1990)
	NS4	CTTCCGTCAATTCCTTTAAG		
Internal transcribed spacer (ITS)	ITS5	GGAAGTAAAAGTCGTAACAAGG		
	ITS4	TCCTCCGCTTATTGATATGC		
Elongation factor-1 alpha (*tef1-α*)	EF1-983F	GCYCCYGGHCAYCGTGAYTTYAT	94 °C 2 min; 36 cycles of 66 °C – 56 °C (touchdown 9 cycles), 94 °C 30 sec, 56 °C 1 min, 72 °C 1 min; 72 °C 10 min; 4 °C on hold	Rehner and Buckley (2005)
	EF1-2218R	ATGACACCRACRGCRACRGTYTG		
RNA polymerase II subunit (*rpb2*)	fRPB2-5F	GAYGAYMGWGATCAYTTYGG	94 °C 3 min; 40 cycles of 94 °C 20 sec, 55 °C 30 sec, 72 °C 1 min; 72 °C 10 min; 4 °C on hold	Liu et al. 1999
	fRPB2-7cR	CCCATRGCTTGYTTRCCCAT		

Phylogenetic analyses

BLASTn (https://blast.ncbi.nlm.nih.gov//Blast.cgi) was used to evaluate closely related strains to our new taxa. Other sequences used in this study were obtained from GenBank referring to Zhang et al. (2012, 2018) and Hongsanan et al. (2020a, 2021) (Table 2). The single gene sequences were viewed using BioEdit v. 7.0.9.0 (Hall 1999). Alignments for each locus were generated with MAFFT v.7 (https://mafft.cbrc.jp/alignment/server/) and manually improved using AliView (Larsson 2014) for maximum alignment and minimum gaps. The final single gene alignments were combined by SequenceMatrix 1.7.8 (Vaidya et al. 2011).

The single locus and combined analyses were carried out for maximum likelihood (ML) and Bayesian posterior probability (BYPP). The ML analyses were carried out using IQ-TREE (Nguyen et al. 2015; Trifinopoulos et al. 2016) on the IQ-TREE web server (http://iqtree.cibiv.univie.ac.at, 30 September 2021) under partitioned models. The best-fit substitution models were determined by WIQ-TREE (Chernomor et al. 2016): SYM+I+G4 for LSU and SSU; TIM+F+I+G4 for *tef1-α*; GTR+F+I+G4 for *rpb2*; TIM2+F+I+G4 for ITS. Ultrafast bootstrap analysis was implemented with 1,000 replicates (Minh et al. 2013; Hoang et al. 2018).

The BYPP analyses were performed in CIPRES (Miller et al. 2010) with MrBayes on XSEDE 3.2.7a (Ronquist et al. 2012). The best nucleotide substitution model for each data partition was evaluated by MrModeltest 2.2 (Nylander 2004). The substitution model GTR+I+G was decided for LSU, SSU, ITS, *tef1-α* and *rpb2* sequences. The Markov chain Monte Carlo (MCMC) sampling approach was used to calculate posterior probabilities (PP) (Rannala and Yang 1996). Six simultaneous Markov chains were run for 10 million generations and trees were sampled every 1,000th generation. The first 20% of trees, representing the burn-in phase of the analyses, were discarded and the remaining trees were used for calculating posterior probabilities (PP) in the majority rule consensus tree.

Table 2. Taxa of Pleosporales used in the phylogenetic analysis with the corresponding GenBank accession numbers. The newly generated strains are indicated in bold. N/A: Not available.

Species names	Strain number	LSU	SSU	ITS	*tef1-α*	*rpb2*
Acrocalymma aquatica	MFLUCC 11-0208	JX276952	JX276953	JX276951	N/A	N/A
Acrocalymma pterocarpi	MFLUCC 17-0926	MK347949	MK347840	MK347732	MK360040	N/A
Acuminatispora palmarum	MFLUCC 18-0264	MH390437	MH390401	NR_163327	MH399248	N/A
	MFLUCC 18-0460	MH390438	MH390402	MN749106	MH399249	N/A
Aigialus grandis	BCC 20000	GU479775	GU479739	N/A	GU479839	N/A
Alternaria alternata	AFTOL ID-1610	DQ678082	KC584507	KF465761	KC584634	KC584375
Amniculicola aquatica	MFLUCC 16-1123	MK106096	MK106108	N/A	MK109800	N/A
Amorocoelophoma cassia	MFLUCC 17-2283	MK347956	NG_065775	MK347739	MK360041	MK434894
Angustimassarina lonicerae	MFLUCC 15-0087	KY496724	N/A	KY496759	N/A	N/A
Anteaglonium parvulum	SMH5223	GQ221909	N/A	N/A	GQ221918	N/A
Aquasubmersa japonica	HHUF 30469	NG_057138	NG_062426	NR_154739	LC194384	LC194421
Aquasubmersa mircensis	MFLUCC 11-0401	NG_042699	NG_061141	JX276954	N/A	N/A
Ascocylindrica marina	MD6011	KT252905	KT252907	N/A	N/A	N/A
	MF416	MK007123	MK007124	N/A	N/A	N/A
Astragalicola vasilyevae	MFLUCC 17-0832	MG828986	MG829098	NR_157504	MG829193	MG829248
Astrosphaeriella fusispora	MFLUCC 10-0555	KT955462	KT955443	N/A	KT955425	KT955413
Atrocalyx acutisporus	KT 2436	LC194341	LC194299	LC194475	LC194386	LC194423
Bahusandhika indica	GUFCC 18001	KF460274	N/A	KF460273	N/A	N/A
Bambusicola bambusae	MFLUCC 11-0614	JX442035	JX442039	JX442031	N/A	KP761718
Berkleasmium crunisia	BCC 17023	DQ280271	N/A	DQ280265	N/A	N/A
Berkleasmium typhae	BCC 12536	DQ280275	N/A	DQ280264	N/A	N/A
Brevicollum hyalosporum	MFLUCC 17-0071	MG602200	MG602202	MG602204	MG739516	N/A
Brevicollum versicolor	HHUF 30591	NG_058716	NG_065124	NR_156335	LC271246	LC271250
Camarosporidiella caraganicola	MFLUCCC 14-0605	KP711381	KP711382	KP711380	N/A	N/A
Camarosporium quaternatum	CPC 31081	NG_064442	KY929123	NR_159756	KY929201	N/A
Camarosporomyces flavigenus	CBS 314.80	GU238076	NG_061093	MH861266	N/A	N/A
Coniothyrium palmarum	CBS 400.71	JX681084	EU754054	MH860184	N/A	KT389592
Corynespora cassiicola	CBS 100822	GU301808	GU296144	N/A	GU349052	GU371742
Corynespora torulosa	CPC 15989	KF777207	N/A	NR_145181	N/A	N/A
Crassiperidium octosporum	MAFF 246406	LC373116	LC373092	LC373104	LC373128	LC373140
Cryptocoryneum japonicum	HHUF 30482	NG_059035	NG_065118	NR_153938	LC096144	LC194438
Cryptocoryneum pseudorilstonei	CBS 113641	NG_059036	LC194322	NR_153941	LC096152	LC194446
Cucurbitaria berberidis	MFLUCC 11-0387	KC506796	KC506800	N/A	N/A	N/A
Cyclothyriella rubronotata	CBS 141486	KX650544	NG_061252	NR_147651	KX650519	KX650574
Cylindroaseptospora leucaenicola	MFLUCC 17-2424	MK347966	MK347856	NR_163333	MK360047	N/A
Dacampia engeliana	Hafellner 72868	KT383791	N/A	N/A	N/A	N/A
Dacampia hookeri	Hafellner 73897	KT383792	N/A	N/A	N/A	N/A
Delitschia chaetomioides	SMH 3253.2	GU390656	N/A	N/A	GU327753	N/A
Delitschia winteri	AFTOL ID-1599	DQ678077	DQ678026	N/A	DQ677922	DQ677975
Dendryphion fluminicola	MFLUCC 17-1689	MG208141	N/A	NR_157490	MG207992	N/A
Dictyocheirospora bannica	KH 332	AB807513	AB797223	LC014543	AB808489	N/A
Dictyosporium elegans	NBRC 32502	DQ018100	DQ018079	DQ018087	N/A	N/A
Didymella exigua	CBS 183.55	MH868977	GU296147	MH857436	N/A	N/A
Didymella rumicicola	CBS 683.79	MH873007	N/A	KT389503	N/A	KT389622
Didymosphaeria rubi-ulmifolii	MFLUCC 14-0023	KJ436586	KJ436588	MK646049	N/A	N/A
Dimorphosporicola tragani	CBS 570.85	KU728536	N/A	KU728497	N/A	N/A
Dothidotthia aceris	MFLUCC 16-1183	MK751816	MK751761	MK751726	N/A	N/A
Fissuroma calami	MFLUCC 13-0836	MF588993	NG_062430	N/A	MF588975	N/A
Flammeascoma bambusae	MFLU 11-0143	NG_059553	KP753952	NR_132915	N/A	N/A
Flavomyces fulophazii	CBS 135761	NG_058131	NG_061191	NR_137960	N/A	N/A
Foliophoma fallens	CBS 161.78	GU238074	GU238215	KY940772	N/A	KC584502
	CBS 284.70	GU238078	GU238218	MH859609	N/A	N/A
Fuscostagonospora cytisi	MFLUCC 16-0622	KY770978	KY770977	N/A	KY770979	N/A

Species names	Strain number	LSU	SSU	ITS	*tef1-α*	*rpb2*
Fuscostagonospora sasae	HHUF 29106	AB807548	AB797258	AB809636	AB808524	N/A
Fusculina eucalypti	CBS 120083	DQ923531	N/A	DQ923531	N/A	N/A
Fusculina eucalyptorum	CBS 145083	MK047499	N/A	NR_161140	N/A	N/A
Halojulella avicenniae	BCC 20173	GU371822	GU371830	N/A	GU371815	GU371786
Halotthia posidoniae	BBH 22481	GU479786	GU479752	N/A	N/A	N/A
Hazslinszkyomyces aloes	CBS 136437	KF777198	N/A	KF777142	N/A	N/A
Helminthosporium velutinum	L131	KY984352	KY984432	KY984352	KY984463	KY984413
Hermatomyces iriomotensis	HHUF 30518	LC194367	LC194325	LC194483	LC194394	LC194449
Hermatomyces tectonae	MFLUCC 14-1140	KU764695	KU712465	KU144917	KU872757	KU712486
Hypsostroma caimitalense	GKM1165	GU385180	N/A	N/A	N/A	N/A
Hypsostroma saxicola	SMH5005	GU385181	N/A	N/A	N/A	N/A
Hysterium angustatum	CBS 123334	FJ161207	N/A	N/A	N/A	N/A
Hysterobrevium smilacis	CBS 114601	FJ161174	FJ161135	N/A	FJ161091	FJ161114
Latorua caligans	CBS 576.65	NG_058180	N/A	N/A	N/A	N/A
Latorua grootfonteinensis	CBS 369.72	NG_058181	N/A	N/A	N/A	N/A
Lentimurispora urniformis	MFLUCC 18-0497	MH179144	MH179160	N/A	MH188055	N/A
Lentithecium clioninum	HHUF 28199	NG_059391	NG_064845	NR_154137	AB808515	N/A
Lentithecium pseudoclioninum	HHUF 29055	NG_059392	NG_064847	AB809633	AB808521	N/A
Lepidosphaeria nicotiae	AFTOL ID-1576	DQ678067	N/A	N/A	DQ677910	DQ677963
Leptosphaeria cichorium	MFLUCC 14-1063	KT454712	KT454728	KT454720	N/A	N/A
Leucaenicola phraeana	MFLUCC 18-0472	MK348003	NG_065784	MK347785	MK360060	MK434867
Libertasomyces myopori	CPC 27354	NG_058241	N/A	KX228281	N/A	N/A
Ligninsphaeria jonesii	MFLUCC 15-0641	NG_059642	N/A	N/A	N/A	N/A
Lindgomyces cigarospora	G619	KX655804	KX655805	KX655794	N/A	N/A
Lindgomyces ingoldianus	ATCC 200398	AB521736	NG_016531	NR_119938	N/A	N/A
Longiostiolum tectonae	MFLUCC 12-0562	KU764700	N/A	KU712447	N/A	N/A
Longipedicellata aptrootii	MFLU 10-0297	KU238894	KU238895	KU238893	KU238892	KU238891
Lophiostoma macrostomum	KT508	AB619010	AB618691	N/A	LC001751	N/A
Lophiotrema eburnoides	KT 1424.1	LC001707	LC001706	LC001709	LC194403	LC194458
Macrodiplodiopsis desmazieri	CBS 140062	NG_058182	N/A	NR_132924	N/A	N/A
Massaria anomia	CBS 59178	GU301839	GU296169	N/A	N/A	GU371769
Massaria inquinans	M19	N/A	HQ599444	HQ599402	HQ599342	HQ599460
Melanomma japonicum	MAFF 239634	NG_060360	NG_065122	NR_154215	LC203367	LC203395
Melanomma pulvis pyrius	CBS 124080	MH874873	GU456302	MH863349	GU456265	GU456350
Misturatosphaeria aurantonotata	GKM 1238	NG_059927	N/A	N/A	GU327761	N/A
Morosphaeria muthupetensis	NFCCI4219	MF614796	MF614797	MF614795	MF614798	N/A
Morosphaeria velatispora	KH221	AB807556	AB797266	LC014572	AB808532	N/A
Multilocularia bambusae	MFLUCC 11-0180	KU693438	KU693442	KU693446	N/A	N/A
Murispora galii	MFLUCC 13-0819	KT709175	KT709182	KT736081	KT709189	N/A
Neocamarosporium goegapense	CPC 23676	KJ869220	N/A	KJ869163	N/A	N/A
Neoconiothyrium persooniae	CBS 143175	MG386094	N/A	MG386041	N/A	N/A
Neomassaria fabacearum	MFLUCC 16-1875	KX524145	NG_061245	N/A	KX524149	N/A
Neomassaria formosana	NTUCC 17-007	MH714756	MH714759	N/A	MH714762	MH714765
Neomassarina thailandica	MFLU 11-0144	NG_059718	N/A	NR154244	N/A	N/A
	MFLUCC 17-1432	MT214467	MT214420	MT214373	N/A	N/A
Neopaucispora rosaecae	MFLUCC 17-0807	MG829033	NG_061293	MG828924	MG829217	N/A
Neophaeosphaeria agaves	CPC 21264	KF777227	N/A	KF777174	N/A	N/A
Neophaeosphaeria filamentosa	CBS 102202	GQ387577	GQ387516	JF740259	GU349084	GU371773
Neophaeosphaeria phragmiticola	KUMCC 16-0216	MG837009	NG_065735	N/A	MG838020	N/A
Neoplatysporoides aloes	CPC 36068	MN567619	N/A	NR_166316	N/A	N/A
Neopyrenochaeta cercidis	MFLUCC 18-2089	MK347932	MK347823	MK347718	N/A	MK434908
Neopyrenochaetopsis hominis	UTHSC DI16 238	LN907381	N/A	LT592923	N/A	LT593061
Neoroussoella bambusae	MFLUCC 11-0124	KJ474839	N/A	KJ474827	KJ474848	KJ474856
Neotestudina rosatii	CBS 690.82	DQ384107	DQ384069	N/A	N/A	N/A
Neoyrenochaeta acicola	CBS 812.95	GQ387602	GQ387541	NR_160055	N/A	LT623271
Nigrograna fuscidula	CBS 141556	KX650550	N/A	NR_147653	KX650525	N/A

Species names	Strain number	LSU	SSU	ITS	*tef1-α*	*rpb2*
Nigrograna mackinnonii	CBS 674.75	GQ387613	NG_061081	NR_132037	KF407986	KF015703
Occultibambusa bambusae	MFLUCC 13-0855	KU863112	N/A	KU940123	KU940193	KU940170
Occultibambusa jonesii	GZCC 16-0117	KY628322	KY628324	N/A	KY814756	KY814758
Parabambusicola bambusina	KH 139	AB807537	AB797247	LC014579	AB808512	N/A
Paradictyoarthrinium aquatica	MFLUCC 16-1116	NG_064501	N/A	NR_158861	N/A	N/A
Paradictyoarthrinium diffractum	MFLUCC 13-0466	KP744498	KP753960	KP744455	N/A	KX437764
Paralophiostoma hysterioides	PUFNI 17617	MT912850	MN582762	MN582758	N/A	MT926117
Parapyrenochaeta protearum	CBS 131315	JQ044453	N/A	JQ044434	N/A	LT717683
Periconia delonicis	MFLUCC 17-2584	NG_068611	NG_065770	N/A	N/A	MK434901
Periconia pseudodigitata	KT 1395	AB807564	AB797274	LC014591	N/A	N/A
Phaeoseptum mali	MFLUCC 17-2108	MK625197	N/A	MK659580	MK647990	MK647991
Phaeoseptum terricola	MFLUCC 10-0102	MH105779	MH105780	MH105778	MH105781	MH105782
Phaeosphaeria oryzae	CBS 110110	KF251689	GQ387530	KF251186	N/A	KF252193
Phaeosphaeriopsis triseptata	MFLUCC 13-0271	KJ522479	KJ522484	KJ522475	MG520919	KJ522485
Plenodomus salvia	MFLUCC 13-0219	KT454717	KT454732	KT454725	N/A	N/A
Pleocatenatium chiangraiense	**MFLUCC 21-0222**	**OL986398**	**N/A**	**OL986396**	**OM240638**	**OM117709**
	MFLUCC 21-0223	**OL986399**	**N/A**	**OL986397**	**OM240637**	**OM117708**
Pleohelicoon richonis	CBS 282.54	N/A	AY856952	MH857332	N/A	N/A
Pleomonodictys descalsii	FMR 12716	KY853522	N/A	KY853461	N/A	N/A
Preussia funiculate	CBS 659.74	GU301864	GU296187	N/A	GU349032	GU371799
Pseudoastrosphaeriella longicolla	MFLUCC 11-0171	KT955476	N/A	N/A	KT955438	KT955420
Pseudoastrosphaeriella thailandensis	MFLUCC 11-0144	KT955478	KT955457	N/A	KT955440	KT955416
Pseudoberkleasmium chiangmaiense	MFLUCC 17-1809	MK131260	N/A	MK131259	MK131261	N/A
Pseudoberkleasmium pandanicola	KUMCC 17-0178	MH260304	MH260344	MH275071	N/A	N/A
Pseudocoleodictyospora tectonae	MFLUCC 12-0385	KU764709	NG_061232	NR_154338	N/A	KU712491
Pseudocoleodictyospora thailandica	MFLUCC 12-0565	KU764701	NG_062417	NR_154337	N/A	KU712494
Pseudodidymosphaeria spartii	MFLUCC 13-0273	KP325436	KP325438	KP325434	N/A	N/A
Pseudopyrenochaeta lycopersici	FMR 15746	EU754205	NG_062728	NR_103581	N/A	LT717680
Pseudopyrenochaeta terretris	FMR 15327	LT623216	N/A	LT623228	N/A	LT623287
Pseudotetraploa longissima	HC 4933	AB524612	AB524471	AB524796	AB524827	N/A
Pseudoxylomyces elegans	KT 2887	AB807598	AB797308	LC014593	AB808576	N/A
Pyrenochaetopsis leptospora	CBS 101635	GQ387627	NG_063097	JF740262	MF795881	LT623282
Pyrenochaetopsis tabarestanensis	IBRC M 30051	KF803343	NG_065034	NR_155636	N/A	N/A
Quadricrura bicornis	yone 153	AB524613	AB524472	AB524797	AB524828	N/A
Quercicola fusiformis	MFLUCC 18-0479	MK348009	MK347898	MK347790	MK360085	MK434864
Quercicola guttulospora	MFLUCC 18-0481	MK348010	MK347899	MK347791	MK360086	N/A
Quixadomyces cearensis	HUEFS 238438	MG970695	N/A	NR_160606	N/A	N/A
Roussoella nitidula	MFLUCC 11-0634	KJ474842	N/A	KJ474834	KJ474851	KJ474858
Salsuginea phoenicis	MFLU 19-0015	MK405280	N/A	N/A	MK404650	N/A
Salsuginea ramicola	KT 2597.2	GU479801	GU479768	N/A	GU479862	GU479834
Seltsamia ulmi	CBS 143002	MF795794	MF795794	MF795794	MF795882	MF795836
Shiraia bambusicola	GZAAS2.629	KC460980	N/A	GQ845415	N/A	N/A
Splanchnonema platani	CBS 222.37	KR909316	KR909318	MH855895	KR909319	KR909322
Sporormia fimetaria	UPS Dissing Gr.81.194	GQ203729	N/A	GQ203769	N/A	N/A
Sporormiella isomera	CBS 166.73	MH872355	N/A	AY943053	N/A	N/A
Stemphylium herbarum	CBS 191.86	GU238160	GU238232	NR_111243	KC584731	DQ247794
Striatiguttula nypae	MFLUCC 18-0265	MK035992	MK035977	MK035969	MK034432	MK034440
Striatiguttula phoenicis	MFLUCC 18-0266	MK035995	MK035980	MK035972	MK034435	MK034442
Sublophiostoma thailandica	MFLUCC 11-0185	KX534216	KX534222	MW136275	KX550080	MW088718
	MFLUCC 11-0207	KX534212	KX534218	MW136257	KX550077	MW088714
Subplenodomus violicola	CBS 306.68	MH870849	GU238231	MH859138	N/A	N/A
Sulcatispora acerina	KT 2982	LC014610	LC014605	LC014597	LC014615	N/A
Sulcatispora berchemiae	KT 1607	AB807534	AB797244	AB809635	AB808509	N/A
Sulcosporium thailandica	MFLUCC 12-0004	KT426563	KT426564	MG520958	N/A	N/A
Teichospora trabicola	C134	KU601591	N/A	KU601591	KU601601	KU601600
Tetraplosphaeria sasicola	KT 563	AB524631	AB524490	AB524807	AB524838	N/A

Species names	Strain number	LSU	SSU	ITS	*tef1-α*	*rpb2*
Thyridaria acaciae	CBS 138873	NG_058127	N/A	KP004469	N/A	N/A
Thyridaria broussonetiae	TB1	KX650568	KX650515	KX650568	KX650539	KX650586
Torula aquatica	MFLUCC 16-1115	MG208146	N/A	MG208167	N/A	MG207977
Torula pluriseptata	MFLUCC 14-0437	KY197855	KY197862	MN061338	KY197875	KY197869
Trematei arundicola	MFLU 16-1275	KX274248	KX274254	KX274241	KX284706	N/A
Trematosphaeria grisea	CBS 332.50	NG_057979	NG_062930	NR_132039	KF015698	KF015720
Trematosphaeria pertusa	CBS 122368	NG_057809	FJ201991	NR_132040	KF015701	FJ795476
Tzeanania taiwanensis	NTUCC 17-006	MH461121	MH461127	MH461124	MH461131	N/A
Wicklowia aquatica	CBS 125634	MH875044	NG_061099	N/A	N/A	N/A
Wicklowia submersa	MFLUCC 18-0373	MK637644	MK637643	N/A	N/A	N/A
Xenopyrenochaetopsis pratorum	CBS 445.81	GU238136	NG_062792	MH861363	N/A	KT389671

Phylogenetic trees were viewed using FigTree v1.4.0 (Rambaut and Drummond 2008) and modified in Microsoft Office PowerPoint 2010 and converted to jpg file using Adobe Photoshop CS6 Extended 10.0 (Adobe Systems, San Jose, CA, USA). The new sequences derived from this study were deposited in GenBank. The final alignment and tree were deposited in TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S29199).

Results

Phylogenetic analyses

Blast searches of LSU, *tef1-α*, *rpb2* and ITS sequences data in NCBI showed that our sequences were related to Acrocalymmaceae, Amorosiaceae, Sporormiaceae and Sublophiostomataceae. One hundred and seventy-six taxa, representing all families in Pleosporales, with *Hysterium angustatum* Alb. & Schwein (CBS 123334) and *Hysterobrevium smilacis* (Schwein.) E. Boehm & C.L. Schoch (CBS 114601) as the outgroups, were selected for the analyses. The final combined dataset consisted of 4,953 characters (LSU: 1–850 bp, SSU: 851–1,851 bp, *tef1-α*: 1,852–2,720 bp, *rpb2*: 2,721–3,701 bp, ITS: 3,702–4,953 bp), including alignment gaps. Among them, 2,336 characters were constant, 608 variable characters were parsimony-uninformative, and 2,009 characters were parsimony informative. The most likely tree (-ln = 98,965.704) is presented (Figure. 1) to show the phylogenetic placement of the newly introduced genus and its relationship with other members in Pleosporales.

Analyses of both ML and BYPP (not shown) yielded almost identical results, and the topology of the trees were similar to previous studies (Zhang et al. 2018; Hongsanan et al. 2020a, 2021). The combined analyses showed that two suborders Massarineae and Pleosporineae were well-supported and formed an upper clade in Pleosporales. Our two newly obtained fungal isolates (MFLUCC 21-0222 and MFLUCC 21-0223) clustered together and formed a distinct clade with maximum support (ML-BS = 100%, BYPP = 1.00) and they grouped with Amorosiaceae, Sporormiaceae and Sublophiostomataceae with weak support.

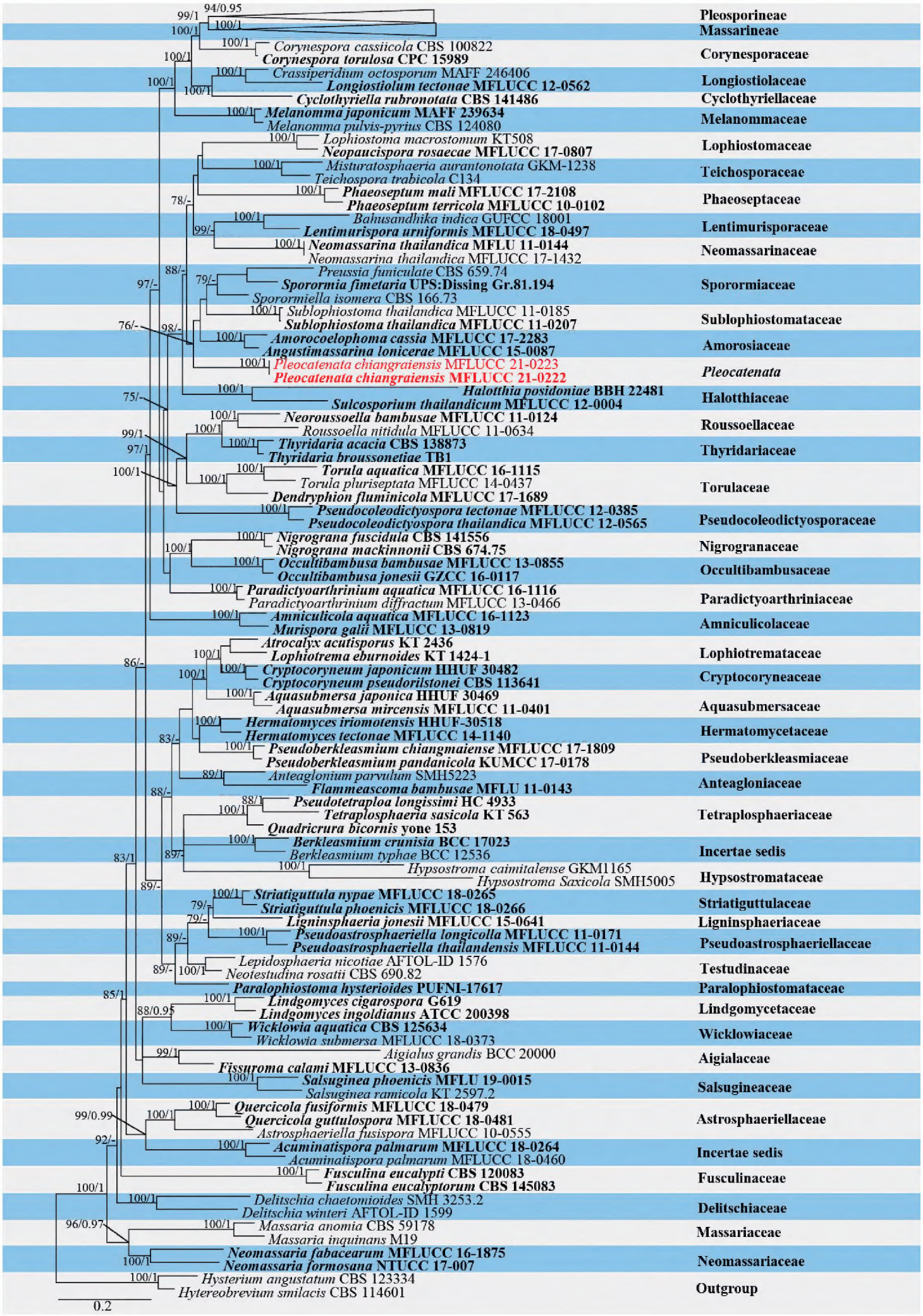

Figure 1. Maximum likelihood tree generated by IQ-Tree, based on analysis of a combined dataset of LSU, SSU, *tef1-α*, *rpb2* and ITS sequence data. Bootstrap support values for ML greater than 75% and Bayesian posterior probabilities greater than 0.95 are given near nodes, respectively. Ex-type strains are in bold, the new isolates are in red.

Taxonomy

Pleocatenata **Y.R. Sun, Yong Wang bis & K.D. Hyde, gen. nov.**
Index Fungorum number: IF559457
Facesoffungi number: FoF 10630

Etymology. "Pleo-" an abbreviation of Pleosporales, the order in which this fungus is classified; "*-catenata*" refers to the catenate conidia of this fungus.

Description. *Saprobic* on decaying twigs in terrestrial habitats. **Asexual morph:** Hyphomycetous. *Colonies* on natural substrate effuse, dark, velvety. *Conidiophores* macronematous, mononematous, straight or slightly curved, cylindrical, unbranched, septate, brown or dark brown. *Conidiogenous cells* monotretic, integrated, terminal, cylindrical, brown to dark brown. *Conidia* catenate, formed in acropetal chains, straight or bent, obclavate, olivaceous to dark brown, multi-euseptate, slightly constricted at septa, distal conidia rounded at apex, truncate at base, intercalary conidia truncate at both ends, with thickened and darkened scars at base or both ends. **Sexual morph:** Undetermined.

Type species. *Pleocatenata chiangraiensis* Y.R. Sun, Yong Wang bis & K.D. Hyde

Notes. The morphology of *Pleocatenata* is distinguished from members in other families in Pleosporales by its tretic conidiogenous cells and catenate, euseptate conidia, and phylogenic analyses indicated it does not belong to any existing families. To avoid establishing a new family with only one species, *Pleocatenata* is introduced as a new genus and assigned to Pleosporales, genera *incertae sedis*. *Pleocatenata* is a monotypic genus reported from terrestrial habitats but without a known sexual morph. Further discovery of other species in *Pleocatenata* or phylogenetic related genera with supported monophyly will determine the familial level of *Pleocatenata*.

Pleocatenata chiangraiensis **Y.R. Sun, Yong Wang bis & K.D. Hyde, sp. nov.**
Index Fungorum number: IF559458
Facesoffungi number: FoF 10631
Fig. 2

Etymology. The epithet referring to the location in which the fungus was collected.

Holotype. MFLU: 22-0002

Description. *Saprobic* on twigs of *Clerodendrum quadriloculare* and *Tarenna stellulata*. **Asexual morph:** Hyphomycetous. *Colonies* on natural substrate effuse, dark, velvety. *Mycelium* immersed, composed of septate, branched, hyaline to subhyaline hyphae. *Conidiophores* macronematous, mononematous, erect, straight or slightly curved, cylindrical, unbranched, robust, 4–6-septate, brown or dark brown, rough, 35–100 μm long, 5.5–8.5 μm wide. *Conidiogenous cells* monotretic, integrated, terminal, determinate, cylindrical, dark brown. *Conidia* catenate, formed in acropetal chains of 2–3, straight or curved, obclavate, olivaceous to brown when young, blackish brown

Figure 2. *Pleocatenata chiangraiensis* (MFLU 22-0002, holotype) **a** host (*Tarenna stellulata*) **b, c** colonies on natural substrate **d, e** conidiophores with conidia **f** conidiogenous cells **g–k** conidia **l** germinated conidium **m, n** colonies on PDA (upper view and lower view). Scale bars: 1 mm (**b**); 100 μm (**c**); 20 μm (**d–l**).

when mature, 5–8-euseptate, slightly constricted at septa, distal conidia rounded at apex, truncate at base, intercalary conidia truncate at both ends, with thickened and darkened scars at base or both ends, 34–70 μm long, 6.5–12 μm at the widest. **Sexual morph:** Unknown.

Culture characteristics. Conidia germinated on PDA within 12 hours at 26 °C. Germ tubes were produced from both ends. Colony reached 20–25 mm diameter after 4 weeks at room temperature on PDA media. Mycelia superficial, irregularly circular, entire edge, dark brown from above, black from below, pigment produced which turns the media reddish brown.

Material examined. Thailand, Chiang Rai Province, Mae Fah Luang University, on twigs of *Tarenna stellulata*, 3 July 2020, Y.R. Sun, MFU5 (MFLU 22-0002, **holotype**, ex-type living culture MFLUCC 21-0222). Thailand, Chiang Rai Province, Medicinal Plants Garden, on twigs of *Clerodendrum quadriloculare*, 7 June 2020, Y.R. Sun, B45 (MFLU 22-0001, living culture MFLUCC 21-0223).

Notes. Two isolates collected from different hosts share similar morphology and clustered together in the phylogenic tree. There are no base pair differences in LSU and *tef1-α* genes between these two isolates. One base pair and two base pair differences (without gaps) are observed in ITS and *rpb2*, respectively. Therefore, the two isolates MFLUCC 21-0222 and MFLUCC 21-0223 are identified as conspecific.

Discussion

Pleocatenata is phylogenetically related to Amorosiaceae, Sporormiaceae, and Sublophiostomataceae in our multi-gene analyses, but their monophyly was not well-supported, indicating their uncertain phylogenetic affinities. No hyphomycetous asexual morph has been reported in Sporormiaceae or Sublophiostomataceae (Hongsanan et al. 2020a, 2021). However, in Amorosiaceae, only two known hyphomycetous genera, *Amorosia* and *Angustimassarina*, are characterized by micronematous to semimacronematous, pale brown conidiophores, monoblastic conidiogenous cells, and single, elongate-clavate conidia (Mantle et al. 2006; Thambugala et al. 2015; Hongsanan et al. 2020a). *Pleocatenata* can be distinguished from these two genera by having monotretic conidiogenous cells and catenate, obclavate conidia.

A recently introduced species, *Corynespora sinensis* Jian Ma, X.G. Zhang & R.F. Castañeda, resembles *Pleocatenata* in its unbranched, cylindrical conidiophores and monotretic, terminal conidiogenous cells that produce catenate, obclavate conidia (Xu et al. 2020). Morphologically, *Corynespora sinensis* is more similar to *P. chiangraiensis* than to the type species of *Corynespora*, *C. cassiicola* (Berk. & M.A. Curtis) C.T. Wei (Wei 1950). Since *Corynespora* (Corynesporascaceae, Pleosporales) is a polyphyletic genus (Schoch et al. 2009; Voglmayr and Jaklitsch 2017), and there is no available sequence data for *C. sinensis*, we presume that *C. sinensis* may belong to *Pleocatenata*. However, due to lack of molecular data, and since morphology-based classification is not reliable for many hyphomycetous genera (Shenoy et al. 2006; Su et al. 2016; Yang

Table 3. Comparison between *Corynespora cassiicola*, *C. sinensis*, and *Pleocatenata chiangraiensis*.

Species	Conidiophores	Conidiogenous cells	Conidia	References
Corynespora cassiicola	Unbranched, cylindrical proliferations, pale to mid brown, up to 9 septate, 110–850 × 4–11 µm	Monotretic, cylindrical, pale to mid brown	Solitary or in chains of 2–6, obclavate to cylindrical, subhyaline to pale olivaceous brown or brown, 4–20 distoseptate, 40–220 × 9–22 µm	Wei 1950
Corynespora sinensis (HJAUP M0156)	Unbranched, cylindrical, brown to dark, 4–8-septate, 53–96.5 × 7–8.5 µm	Monotretic, cylindrical, brown,	In chains of 2, primary conidia obclavate or fusiform, 3(–4)-distoseptate, 31.5–42 × 8–9.5 µm. secondary conidia ellipsoid, 3-distoseptate, 21–28.5 × 8–9.5 µm	Xu et al. 2020
Pleocatenata chiangraiensis (MFLU 21-0222)	Unbranched, cylindrical, brown or dark brown, 4–6-septate, 35–100 × 5.5–8.5 µm	Monotretic, cylindrical, dark brown	In chains of 2–3, obclavate, olivaceous to brown when young, blackish brown when mature, 5–8-euseptate, 34–70 µm × 6.5–12 µm	This study

et al. 2018), we retain the current classification. Sequences of *C. sinensis* are needed to resolve its phylogenetic placement. Detailed morphological comparison among *C. cassiicola*, *C. sinensis* and *P. chiangraiensis* is provided (Table 3).

Pleocatenata is similar to *Sporidesmium sensu stricto*, which is characterized by distinctive, unbranched conidiophores, monoblastic, determinate or proliferating conidiogenous cells, and acrogenous, solitary, transversely septate conidia (Ellis 1958, 1971; Shenoy et al. 2006; Boonmee et al. 2012; Su et al. 2016; Yang et al. 2018). However, *Pleocatenata* is different from *Sporidesmium* by having catenate conidia. Additionally, *Pleocatenata* is phylogenetically distinct from *Sporidesmium*, supporting the introduction of the new genus.

The catenate, obclavate phragmoconidia of *P. chiangraiensis* are similar to capnodendron asexual morph of *Antennulariella* Woron (Antennulariellaceae, Capnodiales) (Hughes 1976, 2000; Seifert et al. 2011). Although sequence data of *Antennulariella* is not available, morphological characters, such as holoblastic conidiogenous cells and branched conidiophores of *Antennulariella*, support its separation from *P. chiangraiensis* (Hughes 1976, 2000; Seifert et al. 2011). *Pleocatenata* is also similar to *Corynesporina* Subram (Pezizomycotina, *incertae sedis*) in having unbranched, robust conidiophores and catenate conidia (Seifert et al. 2011). However, they differ in that the distoseptate conidia form in basipetal chains in *Corynesporina* and euseptate conidia form in acropetal chains in *Pleocatenata*.

Acknowledgements

We would like to thank Dr. Shaun Pennycook for checking the nomenclature. Ya-Ru Sun thanks Mae Fah Luang University for the award of a fee-less scholarship. Ya-Ru Sun also thanks the director of the Mae Fah Luang University Botanical Garden, the botanist Dr. Jantrararuk Tovaranonte for her support. The study was funded by Guizhou Science Technology Department International Cooperation Basic project ([2018]5806), National Natural Science Foundation of China (No.31972222,

31560489), Program of Introducing Talents of Discipline to Universities of China (111 Program, D20023), and Talent project of Guizhou Science and Technology Cooperation Platform ([2017]57885, [2019]5641 and [2020]5001).

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